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● *Original Contribution*

THE EFFECT OF LOW-INTENSITY ULTRASOUND ON BRAIN-DERIVED NEUROTROPIC FACTOR EXPRESSION IN A RAT SCIATIC NERVE CRUSHED INJURY MODEL

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Abstract—Low-intensity ultrasound (LIU) can improve nerve regeneration and functional recovery after peripheral nerve crush injury, but the underlying mechanism is not clear. The objective of this study was to examine the effects of LIU on rat sciatic crush injury and to investigate a possible molecular mechanism. Adult male Sprague-Dawley rats underwent left sciatic nerve crush surgery and were then randomized into two groups: a treatment group that received LIU every other d, and a control group that received sham exposure. Compared with rats in the control group, rats in the treatment group had higher sciatic nerve function indexes, compound muscle action potentials, wet weight ratios of the target muscle and mRNA expression of brain-derived neurotropic factor (BDNF) in the crushed nerve and ipsilateral dorsal root ganglia. Our findings suggest that LIU might promote injured nerve regeneration by stimulating BDNF release. (E-mail: y_wang1111@hotmail.com)

Key Words: **Ultrasound therapy, Peripheral nerve injury, Nerve regeneration, Brain-derived neurotropic factor.**

INTRODUCTION

Peripheral nerve injury can occur in daily life due to mechanical damage resulting from traffic accidents, work-related injuries or surgery. The repair process is slow, even though peripheral nerves have intrinsic regenerative abilities (Gu et al. 2011). If the injured nerve does not recover or regenerate in a timely manner, this may cause temporary or life-long neuronal dysfunctions, such as sensory deficits or motor disturbances, resulting in reduced quality of life and a large economic and social burden. Among various kinds of peripheral nerve injury, crush injury induced by acute compression always leads to axon transection (with intact perineurium and epineurium), which triggers Wallerian degeneration throughout the distal nerve stump and within a small zone at the tip of the proximal stump (Gu et al. 2011; Wang et al. 2013). Thus, the promotion of nerve morphologic and functional recovery deserves much attention and research.

There are many methods of repairing injured nerves and improving nerve regeneration, including surgical operation (*e.g.,* nerve suture, transplantation of autologous nerves or tissue-engineered nerve conduits [Ao et al. 2011; Gu et al.2014]), physical methods (*e.g.,* electric [Elzinga et al. 2015], magnetic field [Rusovan and Kanje 1992], shock wave [Hausner and Nógrádi 2013] or laser stimulation [Sene et al. 2013]) and biological therapy (*e.g.,* administration of neurotrophic factors [Ma et al. 2014; Wenjie et al. 2009], vitamins [Montava et al. 2015] or medications [Wang et al. 2013]). However, some disadvantages limit their clinical application. For example, low-intensity electrical stimulation is invasive and has the potential risk of infection. Autologous nerve transplantation inevitably impairs the function of the donor area. Furthermore, in clinical trials, the administration of recombinant neurotrophic factors has unexpected side effects (Wei et al. 2009). These difficulties urge

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researchers to discover convenient and non-invasive methods of regenerating injured nerves.

Ultrasound, a type of mechanical wave, is primarily used for diagnosis. However, ultrasound is increasingly being used to treat various diseases. For example, high-intensity focused ultrasound can be applied to treat a wide variety of tumors because its high energy markedly increases the temperature of the focused area (Crouzet et al. 2014). Low-intensity ultrasound (LIU) can accelerate injured tissue regeneration and repair bone fractures (Romano et al. 2009) and chronic wounds such as skin or venous ulcers (Beheshti et al. 2014), tendon injury (Hu et al. 2014) and cartilage injury (Khanna et al. 2009). LIU could also contribute to the recovery of peripheral nerve injury (Chen et al. 2010; Raso et al. 2005). The mechanism by which LIU induces a positive biological response could be an interaction among the thermal effects and mechanical effects of ultrasound. However, local heating caused by intensities below 500 mW/cm^2 is minimal (Fowlkes and Holland 2011). And cavitation effects are unlikely due to the high threshold for cavitation in tissue. Therefore, the mechanical effect of ultrasound may be the principal mechanism by which LIU promotes peripheral nerve regeneration (Mourad et al. 2001), possibly by causing the movement of material within cells, stimulating cytomembrane diffusion, promoting metabolism, strengthening blood circulation and improving tissue nutrition. However, the molecular mechanism of ultrasound is not yet clear.

In this study, we used a rat sciatic nerve crush injury model to evaluate the effects of LIU on peripheral nerve regeneration. After treatment with LIU, CatWalk gait analysis, electrophysiological evaluation, transmission electron microscopy and calculation of the wet weight ratio of the target muscle were performed to assess the histologic and functional recovery of injured sciatic nerves (Bozkurt et al. 2011; Wolthers et al. 2005). Furthermore, quantitative real-time polymerase chain reaction (PCR) was used to evaluate the gene expression of brain-derived neurotropic factor (BDNF) in crushed nerves and ipsilateral dorsal root ganglia (DRG) at L4-L6 to investigate a possible mechanism of the effect of LIU on injured nerves.

MATERIALS AND METHODS

Experimental groups

All procedures involving animals were carried out according to the US National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Administration Committee of Experimental Animals in Jiangsu Province, China. Adult male Sprague-Dawley rats weighing 200–240 g were provided by the Experimental Animal Center of Nantong University. The left sciatic nerve of all experimental rats was crushed at 6–8 wk of age, and rats were divided randomly into two groups (n = 40 rats per group): a treatment group that received LIU every other d, and a control group that received sham exposure. Within each group, a random sample of 10 rats was evaluated each wk after surgery for 4 wk; five rats were used for functional and histologic evaluation and five rats were used for gene expression and electron microscopy analysis. All rats were kept under controlled temperature (22–24°C) and humidity (40%–70%) conditions with a 12:12 h light:-dark cycle. The experiments lasted 4 wk following the crush injury.

Surgery

Rats were deeply anesthetized at the dosage of 0.3 mL/100 g weight by an intra-peritoneal injection of a compound agent, which was 4.25 g chloral hydrate, 2.12 g magnesium sulfate, 0.886 g pentobarbital sodium, 14.25 mL absolute ethyl alcohol, 33.8 mL 1, 2 -propylene-glycol and double-distilled water to a total volume of 100 mL. The sciatic nerve was exposed by a longitudinal incision on the mid-thigh of the left hind limb, and a 3-mm section of the nerve was crushed for 30 s with hemostatic forceps to completely rupture nerve fibers. The crush site was marked with a 8-0 nylon suture, and the surgical incision was closed. Each rat received a daily intraperitoneal injection of 40,000 IU penicillin sodium for 3 d to prevent infection.

Ultrasound protocol

Starting 72 h after surgery, rats in the treatment group were fixed to a wooden table on their side, with a water bag between the rats' side and the table, and received ultrasonic irradiation at the incision site every other d using a portable ultrasonic treatment apparatus with an unfocused circular probe with an active area of 4 cm^2 (DJO, Chattanooga, TN, USA). The circular probe was placed vertically on the surgical incision during treatment to ensure the crushed site of the sciatic nerve was irradiated. Aquasonic gel was employed as a coupling medium between the transducer and skin during treatment. The radiation parameters were as follows: continuous wave mode, 1 MHz frequency, 0.2 W/cm^2 intensity (spatial peak and temporal averaged acoustic intensity) and 1 min/d treatment duration. Rats in the control group were handled the same as rats in the treatment group, but the ultrasonic treatment apparatus was turned off (Fig. 1).

CatWalk gait analysis

CatWalk gait analysis is an automated quantitative test for objectively detecting dynamic and static gait changes associated with adult rat sciatic nerve injury